GLOBAL JOURNAL OF AGRICULTURAL SCIENCES VOL 1, NO. 1, JANUARY 2002: 41-47

INFLUENCE OF POULTRY MANURE AMENDMENT ON BIOREMEDIAL ACTIVITY OF PETROLEUM OIL POLLUTED SOIL AND EARLY COWPEA GROWTH.

C. S. IWUOHA ODOEMENA

(Received 31 October 2000; Revision accepted 6 June, 2001)

ABSTRACT

Bioremedial ability of poultry manure on crude-oil-polluted soil and the effect on the seed germination as well as early growth rate of cowpea (*Vigna sinsnsis)* on the soil were studied. The results showed that addition of poultry manure led to favourable increase in pH of the polluted soil. The total nitrogen and phosphorus content as well as the exchangeable cations levels of the contaminated soil were significantly ($P \leq 0.05$) increased. Carbon dioxide production was significantly ($p \leq 0.05$) and positively correlated with increased concentration of poultry manure. Results on the cowpea germination and plant growth of the treated soil showed that increased levels of poultry manure treatment at 0, 250, 500, 750 and 1000g on 4kg soil, polluted with crude –oil (2L) increased the cumulative germination percentage (CGP) and yield components of the plant. Poultry manure treatment at 1000g and 750g gave the highest cumulative germination percentages (CGP) of 83.3 and 75.0% with coefficient of germination velocity (CGV) of 0.075 and 0.069 respectively. Also, there was a significant ($P < 0.05$) reduction in the leaf abscission with increased poultry manure level. The result findings have proved PM to be potentially suitable for ameliorating oil polluted soil.

Key Words: Poultry Manure, oil-polluted-soil, Cowpea growth.

INTRODUCTION

Oil contaminated soils have posed severe difficulties for agricultural crop production in oil-producing and processing areas in Nigeria. These problems arise from induced soil infertility due to hydrocarbon accumulation. Udo and Fayemi (1975) reported that pollution level as low as 1% crude oil in soil suppressed the growth of *Zea mays*. Southwick (1976) observed that seed germination was inhibited at a high pollution level and retarded at a low level.

Hydrocarbon polluted soils are amenable to bioremediation as micro-organisms capable of degrading petroleum hydrocarbons are present (Jones and Edington, 1968). However, the biodegradation ability of these micro-organisms may not contribute significantly to enhanced crop yield in oil-polluted soils. This situation has been associated with high salinity and reduced mineralization level of the soil, (Rhykerd *et al.*, 1995).

Biological remediation of organic chemical contaminated soils is an alternative treatment technology that can often meet the goal and can also ameliorate soil infertility. Holliday and Deul (1994) reported a higher level of hydrocarbon degradation in soils amended with organic nutrients than in soils with low nutrient level. Information on the on-site biodegradation and detoxification of parent organic compounds such as 'petroleum oil, may be obtained using chemical and bioassay analyses (Brubaker and Exner, 1988), although this method is very expensive and time consuming. Organic waste materials such as poultry manure may enhance bioremedial

C. S. IWUOHA ODOEMENA, Department of Botany and Microbiology, University of Uyo, Uyo, Nigeria.

activities of crude-oil contaminated soils at a cheaper and faster rate, hence this study was carried out. Secondly, there is paucity of information on the accelerated bioremediation activity of oil-polluted soil with poultry manure (PM) amendment, besides assessing the soil productivity, with an indicator plant.

MATERIALS AND METHOD

Sample Collection

Sterilized loamy ultisol and poultry manure (PM) were obtained from the Departments of Soil and Animal Sciences of the University of Uyo, experimental and commercial farms respectively. Mature Cowpea (*Vigna sinensis)* seeds of local variety type were obtained from Horticultural Research Institute of Nigeria, Okigwe, Imo State. The crude oil sample was collected from the Mobil Nigeria Nigeria Unlimited Qua Ibeno Eket, Akwa Ibom State.

Physicochemical Analysis Of The Soil Samples

Soil particle analysis was carried out using the hydrometer method (Day, 1956). Soil pH was measured in 1:1 soil to water suspension using a pH meter with a glass electrode. Organic carbon was determined by the wet dichromate oxidation (Allison, 1965). The available phosphorus was extracted and estimated with Bray 1 reagent, calcium and magnesium were determined by titration with EDTA (Jackson, 1959) while exchangeable K and Na were read from a Gallenkamp flame analyzer. Nitrogen was determined by the kjeldahl method.

Estimation Of Co_2 Evolution during Bioremediation

The rate of crude oil biodegradation in the PM amended soil samples were monitored by CO_2 evolution technique (Caxufield, 1961). Twenty grams of the contaminated soil were weighed into screw-capped bottles. Different concentrations of the poultry manure amendments were prepared by dissolving 1.0g, 2.0g, 3.0g, 4.0g and 0g of the PM in 1 litre of distilled water. These gave concentrations of 1000ppm, 2000ppm, 3000ppm and 4000ppm with 0ppm as the control (unamended soil). 1ml of the different PM concentrations were added into each of the screw-capped bottles containing 20g of the oil-contaminated-soil. The bottles were then filled to full capacity with distilled water by adding a mixture of 1.0g of barium peroxide and 10ml of distilled water contained in a vial into each of the screw-capped bottles and tightly closed. The bottles were later incubated at room temperature (28 ± 2°C). At intervals of 1, 7, 14, 21, and 28 days the vials were withdrawn and replaced with fresh sets at 7 days intervals for 28 days.

Planting Media Preparation

Soil pollution consisted of 2L of crude oil, thoroughly mixed with 4kg of loamy soil and left for 21 days. At the 22nd day, O (control), 250, 500, 750) and 1000g of PM were thoroughly mixed with the 4kg oil-polluted-soil and allowed to incubate at 27 ± 2°C for 7 days. Each amended polluted soil sample was placed in perforated plastic bag (18cm in diameter). The perforation of the bag was to enhance drainage of excess water.

Germination Study

Five cowpea seeds were sown in each of the plastic bags containing polluted soil amended with PM. Each treatment was replicated 3 times and watered as the need arose. Seed germination was indexed by the protrusion of 0.5 to 2.5mm of plumule at the time of observation (Odoemena, 1988). The germination rate was observed every 2 days and calculated as coefficient of germination velocity (CGV) according to the method of Hartman and Kester (1964). The cumulative germination percentage (CGP) was determined for each treatment after 10 days of the germination study.

Growth Analysis

The seedlings were allowed to grow for 35 days before harvesting. Three cowpea plants from each treatment were uprooted and rinsed in slow running tap water to remove soil particles. The plant height was measured. The leaf area of the plant was determined according to the method of Hoyt and Bradfield (1962) and the number of leaf abscission was recorded. The fresh masses of the plant component parts as well as the whole plant

Table 1: Physicochemical properties of oil contaminated soil amended with poultry manure

Soil properties	Garden soil unpolluted (control)	Garden soil Polluted with Petroleum oil	Polluted Garden soil amended with poultry manure
Sand	78..21	78.19	78.23
Silt (%)	60.82	5.15	7.30
Clay (%)	16.25	16.23	16.22
PH	6.60	5.28	6.50
Organic Carbon (%)	1.17	5.13	21.0
Total Nitrogen (%)	0.18	0.09	1.16
Phosphorus (%)	13.10	5.20	18.34
C/N ratio	8.62	57.0	3.86
Exchangeable cations (cmol kg^{-1} soil)			
K	0.34	3.50	3.80
Ca	1.90	2.90	4.50
Mg	0.82	2.61	3.46
Na	0.41	0.53	1.33

The accelerated rate of biodegradation of the crude oil in the soil with PM treatment was monitored by CO_2 production (Table 2). The result revealed that higher concentration of PM induced higher carbon dioxide evolution with a peak production at the 6 week study period.

Table 2: Cumulative carbon dioxide evolution form crude oil-polluted soil amended with poultry manure (dm^3/2 weeks)

	Poultry manure concentration (ppm)				
Time Weeks	0	1000	2000	3000	4000
1	38.3±0.75	62.4±0.50	71.5±1.20	80.3±0.80	101.2±2.15
2	86.2±1.13	148.6±1.12	165.1±1.80	183.01.90	197.1±1.85
4	169.5±1.05	226.8±2.25	245.32.10	273.2±2.50	326.4±3.50
6	186.7±2.15	241.2±3.10	266.2±2.54	295.1±2.20	351.1±2.36
8	190.3±3.25	196.8±1.20	232.4±1.23	251.92.55	281.7±1.15

Data are mean ± standard deviation of triplicate determination ± standard deviation

were measured. The weighed seedlings were dried in an oven maintained at 80°C for 2 days for determination of dry matter accumulation. The difference between the fresh and dry masses of the plant is taken as the moisture content of the seedling (Meyer *et al.*, 1973).

The root-shoot dry mass ratio was calculated following the method of Varma and Poonia (1979). The leaf area ratio (LAR) calculated as the ratio of the area to the total plant dry mass, leaf mass ratio (LMR), stem mass ratio (SMR) and root mass ratio (RMR) defined as the dry mass of each component of the plant part to the total dry mass of the whole plant, were determined (Hoyt and Bradfield, 1962). All the data obtained were subjected to a two way analysis of variance (ANOVA) according to the method of Sokal and Rohlf (1969) and used to estimate the significant growth parameters.

RESULTS

Physicochemical properties of the poultry manure amended oil-polluted soil as well as that of unamended polluted and unpolluted soils are shown in table 1. The result shows that oil pollution reduced the soil pH level from 6.60 to 5.20 while PM amendment normalized the pH level. The result clearly shows that PM amendment on crude-oil-polluted soil gave higher and significant ($p < 0.05$) total Nitrogen and Phosphorus percentages as well as exchangeable cations levels than those of unpolluted and polluted unamended soils.

The results of bioremedial effect of poultry manure for restoration of oil-polluted soil productivity using cowpea as an indicator plant are shown in fig. 1, table 3 and 4. The cumulative germination percentage of cowpea seeds in oil polluted soil amended with 750 and 1000g PM were 75% and 83.3% respectively, which are significantly ($p \leq 0.05$) higher than those of 500g (41.67% and 250g (15.80%) PM amendment (fig. 1). The treatment without PM gave no germination at the end of the period for germination study. The coefficient of germination velocity (CGV) increased with increase in PM amendment of the soil. The increases in CGV between 250g (0.0476) and 500g, (0.0555) PM treatment were not significantly ($p \leq 0.05$) different from others, but were different from those of 750, (0.0692) and 1000g (0.0746).

Results on the growth characteristics examined, indicate a general increase a general increase due to PM treatment (Table 3). The total dry mater accumulations of the leaf, stem,

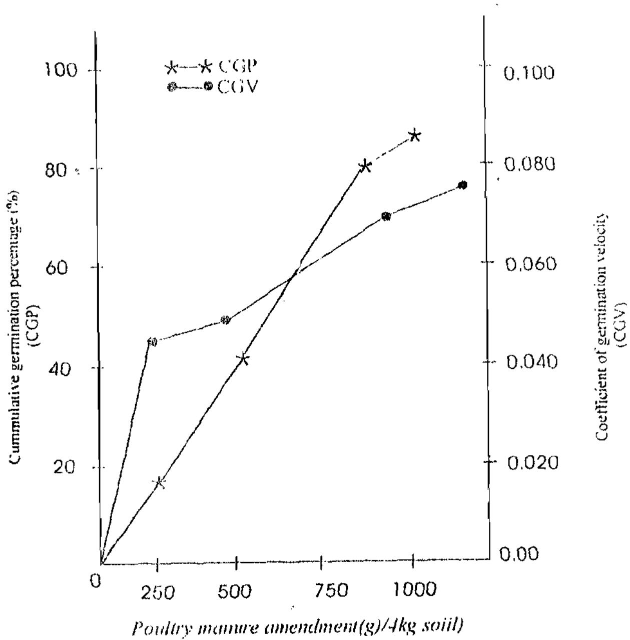

Fig. 1 Effect of poultry manure amendment on cumulative seed germination percentage and coefficient of germination velocity of cowpea grown in crude oil polluted soil

root and whole plant as well as the fresh masses were significantly ($p \leq 0.05$) higher in 1000g PM treatment than those treated with 750 or 500g PM. Leaf abscission decreased with increases in PM addition (Table 3). The root-shoot ratio of the crop was highest at 500g PM treatment and least at 250g. the moisture content of the plant also increased with increases in PM treatment.

The results of individual yield components calculated as the ratio of mass of each part of the plant to the today dry mass of the whole plant are show in table 4 as LAR, LMR, SMR and RMR. The values of LAR significantly ($p \leq 0.05$) increased from 3.62cm2 g-1 dry mass at 250g PM addition to 5.92cm2 g-1 dry mass at 500g PM and suddenly dropped to 4.11 and 3.36cm2 g-1 dry mass at 750 and 100g PM treatments, respectively. The LMR increased with increases in PM treatment showing a significant difference between 250 and 500g PM amendment. The SMR decreased with increase in PM treatment, whereas the RMR increased significantly ($p \leq 0.05$) from 250 to 500g PM treatments and gradually dropped with further increased PM treatment.

DISCUSSION

The interest in this study is focused on the possibility of using simple and cheap biotechnologically based methods to restore soil fertility for effective crop growth and productivity after hydrocarbon pollution.

The normalization ability of PM treatment on the polluted soil pH level, met with the optimal pH range for hydrocarbon degradation and microbial activity (Bindra and Zester, 1978). The higher and significant percentages of total Nitrogen, phosphorus and exchangeable cations recorded on soil analysis could be corroborated with the report of Mbagwu (1985) on poultry manure as a rich source of N, P, K, Ca, Mg and Na. The variations in the test soil particles are not at variance with the report of Essien *et al.*, (1999). The peak CO_2 evolution at the 6 week period of the experiment could depict the end period of biodegradation of the oil

Table 3: Effect of different levels of poultry manure amendment on the physical and structural characteristics of Cowpea (*vigna sinensis*) grown in crude-oil-polluted soil

	Poultry manure amendment in grammes per 4kg soil (gkg^{-1} soil)					
Growth parameters	0	250	500	750	1000	Mean
Leaves						
Fresh mass (g)	ND	2.75±0.35	8.27±1.25	11.45±2.15	14.56±1.23	9.26±1.25
Dry mass (g)	"	0.120.01	0.35±0.02	0.94±0.20	1.52±0.10	0.73±0.17
Lea area (cm^2)	"	4.16±1.10	9.83±1.25	13.18±1.85	16.32±1.50	10.87±1.64
Leaf Abscision	"	5.03±0.50	3.25±0.13	2.75±0.12	1.500.13	3.13±0.21
Stem						
Fresh mass (g)	"	5.20±0.15	12.15±1.20	16.03±1.25	22.06±2.10	13.86±1.50
Dry mass (g)	"	0.95±0.03	1.02±0.30	1.92±0.35	2.75±0.20	1.66±0.25
Root						
Root length cm^2)	"	9.45±0.62	12.072.50	15.04±1.22	17.51±1.25	3.52±1.30
Fresh mass (g)	"	0.52±0.05	0.95±0.02	1.50±0.03	2.05v0.30	1.26±0.20
Dry mass (g)	"	0.08±0.02	0.29±0.01	0.35±0.05	0.59±0.02	0.33±0.03
Whole Plant						
Plant height (cm)	"	35.34±2.10	85.661.75	92.12±1.20	115.07±2.50	82.05±1.40
Fresh mass (g)	"	8.47±0.75	21.37±1.50	28.98±1.10	38.56±1.20	24.37±1.02
Dry mass (g)	"	1.15±0.03	1.66.280.02	3.21±0.32	4.86±0.15	2.27±0.03
Root/shoot dry mass ratio	"	0.08±0.03	0.28±0.02	0.18±0.03	0.21±0.05	0.19±0.01
Moisture content(g)	"	7.32±0.50	19.71±35	27.77±1.62	33.81±1.70	21.65±1.20

Data are mean of 3 determinations ± standard deviation
ND – Not determined (No plant growth).

Table 4: Effect of various levels of poultry manure amendment (gkg-4 soil) on leaf area ratio (LAR), leaf mass ration (LMR), stem mass ratio (SMR), root mass ratio (SMR), and Root mass ratio (RMR).

Poultry manure Amendment (g/4kg soil)	Leaf area ratio (LAR) (cm2g^{-1} dry mass)	Leaf mass ratio (LMR) (gg^{-1})	Stem mass ratio (SMR) (gg^{-1})	Root mass ratio (RMR) (gg^{-1})
0	N/D	ND	ND	ND
250	3.62±0.12	0.10±0.01	0.83±0.03	0.07±0.00
500	5.92±0.06	0.21±0.01	0.61±0.01	0.17±0.02
750	4.11±0.15	0.29±0.02	0.60±0.02	0.11±0.01
1000	3.26±0.12	0.31±0.01	0.57±0.03	0.12±0.02

Each data is a mean of 3 determinations ± standard deviation
ND = Not determined (no plant growth)

The failure of seeds grown in unamended polluted soil to germinate, confirms that seeds undergo dormancy or are killed as a result of soil pollution (Schewendinger, 1968, Udo and Fayemi, 1975; udo and Oputa, 1984). The increased cumulative germination percentage (CGP) and coefficient of germination velocity (CGV) achieved with increased levels of PM treatment on the oil-polluted soil reveal the bioremediation potential of the organic manure in ameliorating condition of the soil and dissipating the toxicity of spilled hydrocarbon compounds.

The significant increases ($p \leq 0.05$) in the cowpea height, leaf area and fresh and dry matter accumulations in the various plant components and the whole plant coupled with the reduction in leaf abscission, as a result of increased PM levels, indicate increase in physiological and biochemical responses of the plant due to the amendments.

This finding is in line with the report of Amakiri and Onofeghare (1983). Also, addition of oil to soil, stimulates carbon dioxide production, which could increase the soil acidity and in turn retard plant physiological processes (Rhykerd *et al.,* 1995).

The increased LAR values of cowpea obtained from 250g to 500g PM treatment and the subsequent drop in their values at 750 or 1000g PM treatments indicate a compensatory mechanism due to heavy shading of the leaves. Also, the increased LMR value with increase in PM treatment is attributed to the reduction in leaf abscission. The decrease in SMR above 250g and RMR at 750g must have been affected directly by the production and partitioning of the photosynthetic products (Boyd and Murray, 1982). The increase in moisture content of the plant with increased PM levels may suggest nutrient and moisture stresses due to oil pollution. Similar observations were reported by Udo and Oputa (1984).

The reduction in RMR, LAR and SMR values as the PM treatment increased from 500g to 1000g may be due to increased dry matter accumulation of the whole plant. The increased value of LMR with increased PM addition could be a compensatory mechanism, possibly as a result of impaired nutrient and moisture absorption by the plant at lower PM treatment.

In conclusion PM additive in oil-polluted soils is highly recommended for enhanced bioremedial and restoration of contaminated soils. The significant differences ($p \leq 0.05$) in carbon dioxide production between time and between PM levels and cowpea component yields are clear indications that PM is a bioremedial booster.

REFERENCES

Allison, C. E., 1965. Organic Carbon: In Methods of Soil Analysis. Edited by Black C. A. American Scotty of Agronomy. Madison Wisconcin 1367 - 1378.

Amakiri, J. O. and Onofeghare, F. A., 1983. Effect of crude oil pollution on the growth of *Zea mays, Abelmoschus esculentus* and *Capsicum frutescens. Oil and Petrochem Pollutes.* 1 : 199 - 205

Antai, S. P. and Mgbomo W., 1989. Distribution of hydrocarbon utilising bacteria in oil spill areas. Macrobios letters 40: 137 – 143.

Atlas, R. M. 1995. Petroleum biodgradation and oil spill bioremediation. Mar. Pollu. Bull. 31 (4 - 12): 178 - 182

Bindra, J. S. and Zester, I. P., 1978. Biodegradation of refinery oil wastes through land application. Chevron Research Company, Richomond, California, presented at Aiche meeting Nov. 1977, San Francisco, CA.

Booyd, J. W. and Murray, D. S., 1982. Effects of shade on silver leaf weight. Weed sci. 30, 259 – 264.

Brubaker, G. R. and Exner, H. H., 1988. Bioremediation of chemical spills pp 163-171 *In: G. S.* Omenn Environmental Biotechnology – Reducing Risks from environmental chemicals through Biotechnology. Plenum Press, New York.

Caxufield, A. H., 1961. Simple technique for determining mineralisation of carbon during incubation of soil treated with organic material. Plant and Soil 14: 90 – 93.

Day, P. R., 1956. Particle fractionation and particle size analysis. In: Methods of soil analysis part I. Physical and mineralogical properties, including statistics of measurement and sampling. C. A. Black *et al.*, (eds). Agronomy 9: 545 – 567.

Essien, J. P., Udosen E. D. and Ubom, R. M., 1999.

Seasonality in fungistasis of petroleum contaminated Nigerian coastal soil. Global Journal of Pure and Applied Sciences 5 (4): 519 – 524.

Hartman, H. T. and Kester, D. E. 1964. Plant Propagation Principle and Practice. U.S.A Prentice Hall, P. 89 – 115.

Holliday, G. H. and Deuel, L. E. 1994. Soil remediation for petroleum extraction industry. Soil Remediation seminar Text. September. American Petroleum Institute Publication. Washington D. C

Hoyt, P and Bradfield, R., 1962. Effects or varying leaf area by defoliation and plant density on dry matter production of corn. Agronomy Jour. 54: 523 – 525.

Jackson, M. L. 1958. Soil Chemical analysis. Prentice Hall, Englewood cliffs, N. J.

Jones, J. G. and Edington, M. A., 1968 An ecological survey of hydrocarbon oxidizing microorganisms. J. Gen. Microbio. 52: 381 – 390.

Mbagwu, J. S. C., 1985. Subsoil productivity of an Ultisol in Nigeria as affected by organic wastes and inorganic fertilizer amendments. Soil Science *140* (6): 436 – 441.

Meyer, B. S., Anderson, D. B. and Bohining, R. H. 1973. Growth Correlations and growth periodicity *In:* Introduction to Plant Physiol. D. Van Nostrand company, New York P. 507 – 529.

Odoemena, C. S., 1988. Breaking of Seed Coat dormancy in a medicinal plant *Tetrapleura tetraptera*. Jour. Agric. Sci. 111 (2): 393 – 394.

Phykerd, R. L., Weaver, R. W. and McLinnes, K. J 1995. Influence of salinity on bioremediation of oil in soil. Environ. Pollut. 90 (1): 127 - 130

Schewndinger, R. B., 1968. Reclamation of soil contaminated with oil. Jour. Inst. Petrol. 54: 182 – 197.

Sokal, R. R. and Rohalf, F. J., 1969. Introduction to Biostatistics. W. H. Freeman and company. San Francisco. P. 368.

Udo, E. J and Fayemi, A. A., 1975. The effect of oil pollution of soil on germination, Growth and nutrient uptake of corn (*Zea mays*) Jour. Environ, Qual. 4: 537 – 540.

Udo, E. J. and Oputa, C. O., 1984. Some studies on the effect of crude oil pollution of soil on plant [illegible] *Jour. Biol.* Appl. Chem. 3: 26 – 29.

Varma, S. K. and Poonia, S. L., 1979. Germination and early seedling growth of Pearl Milet (*Pennistum typhoides L.*) as affected by salinity. Indian J. Plant Physiol. 26: 142 – 146.